Plasma Leptin Is Not Associated With Insulin Resistance and Proinsulin in Non-diabetic South Asian Indians

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In an earlier study, we observed only a weak association between plasma insulin (non-specific assay) and leptin in South Asian Indians. This was in contrast to the observations in many other ethnic groups. With the availability of measurements of specific insulin (SI) and proinsulin (PI) in the same study group, we have reanalysed the data to look for possible correlation of leptin with proinsulin and with insulin resistance calculated from the fasting values of specific insulin and glucose using the HOMA model. Subjects with normoglycaemia ($n = 117$) and impaired glucose tolerance ($n = 27$, WHO criteria) were included in the analysis. Leptin values were higher in women. Multiple linear regression analysis showed that the variations in leptin concentrations in men were associated with BMI, WHR, and 2 h SI values ($R^2 = 56.2$ %) while fasting SI and proinsulin concentrations had no significant association. In women BMI and age showed a significant association with serum leptin values ($R^2 = 40.1$ %). Univariate and multivariate analyses using insulin resistance as the dependent variable showed that it had no association with leptin in both genders. Leptin had no correlation with proinsulin also. This study confirmed that in Asian Indians the association between plasma leptin and insulin concentrations is weak and that leptin has no influence on insulin resistance. Proinsulin and leptin are also not correlated in this population. Insulin resistance shows correlation with the β-cell function both in men and women. © **1998 John Wiley & Sons, Ltd.**

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Introduction

A strong positive association between plasma leptin and insulin has been demonstrated in several ethnic groups.[1–4] In an earlier report, we demonstrated that, in South Asian Indians, plasma leptin levels are similar to those of other populations but that the association between leptin and plasma insulin is weak, despite the presence of high insulin concentrations.[5] In that analysis, we could use only the non-specific insulin measurements, which cross-react with proinsulin and its split products. With the availability of measurements of specific insulin and proinsulin using specific RIA procedures, we have looked at the possible correlation of leptin with proinsulin and also with insulin resistance (IR) derived from the fasting specific insulin values using the homeostasis model assessment model (HOMA)[6] in our population. HOMA has been validated as a useful method of assessing insulin resistance (IR) and β-cell function (BF) using the fasting plasma glucose and insulin response.[7–9]

Study Subjects and Methods

One hundred and forty-four adults aged ≥20 years with 2 h plasma glucose (2 h PG) <11.1 mmol l^{-1} after a 75 g glucose load were included in this analysis. There were 117 with normoglycaemia (2 h PG <7.8 mmol l^{-1}, NGT) and 27 with impaired glucose tolerance (2 h PG >7.8 to <11.1 mmol l^{-1}, IGT) according to current WHO criteria.[10] We included IGT also for the analysis (a) to obtain a wide range of insulin resistance and (b) because leptin values were not different in NGT and IGT.[5] Details of the sample selection and assay procedures have beeen described.[5]

Plasma samples were stored at –70 °C prior to analysis. Leptin, specific insulin (SI), and proinsulin (PI) were estimated with these samples by using the respective RIA kits supplied by LINCO Research Inc., St Louis, Mo., USA. Leptin was estimated in the fasting sample and the SI and PI were estimated in the fasting and 2 h samples of GTT.

Monospecific antibodies were used in the assays. The

Abbreviations: BF beta cell function, IR insulin resistance, PI proinsulin, SI specific insulin

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kit for specific insulin uses an antibody that reacts with the free NH_2-terminal of the A-Chain of Insulin. Intact human proinsulin and Des 31, 32 human proinsulin do not cross-react significantly (<0.2%). Although the cross-reactivity with Des 64,65 proinsulin is much higher (approximately 76 %), this product of proinsulin comprises <5 % of all proinsulin. For specific insulin, the lowest detection limit was 14.4 pmol l^{-1}. LINCO's human proinsulin RIA utilizes an antibody made specifically against human proinsulin which recognizes a specific epitope formed by intact A-Chain C-peptide junction. Under non-equilibrium conditions, A-Chain C-peptide junctional cleaved forms of proinsulin are <1 % as potent as intact proinsulin, whereas B-Chain C-peptide junctional cleaved forms, such as des 31,32 proinsulin, have a cross-reactivity >95 %. Because des 31,32 is the major circulating form of split proinsulin (approximately 95 %), the proinsulin RIA used in this study provides an estimate of total concentration of proinsulin (intact + B-C-Junctional cleaved forms) in plasma. The lowest detection limit of proinsulin was 2 pmol l^{-1} and the intra- and inter-assay variations were <7 % to 11 % for both assays.

For the leptin assay, the antibody in the RIA was a polyclonal antibody raised in rabbits against highly purified recombinant human leptin.[11] Both the calibrators and ^{125}I-labelled tracer were prepared with recombinant human leptin. The sensitivity of the assay was 0.5 ng ml^{-1}. The intra- and inter-assay CV were 4.2 % and 7 %, respectively.

Insulin resistance (IR) and β-cell function index were derived using the HOMA calculation.[6] The formulae used are given below:

$$\text{Insulin resistance (HOMA IR)} = \text{Fasting insulin } (\mu\text{u ml}^{-1}) \times \frac{\text{Fasting glucose mmol l}^{-1}}{22.5}$$

$$\text{Beta-cell function (HOMA BF)} = \frac{20 \times \text{Fasting insulin } (\mu\text{u ml}^{-1})}{\text{Fasting glucose (mmol l}^{-1}) - 3.5}$$

Statistical Analysis

Group means were compared by unpaired *t*-test or by one way ANOVA as relevant. Leptin, PI, SI, IR, and BF values were log transformed prior to analysis. For these parameters, geometric means and confidence intervals are reported. Mean and SD of other variables are reported (Table 1). In order to find out the linear relationship of IR with individual independent variables, partial correlations were evaluated. In this test, the association of one variable with IR was evaluated keeping the other variables constant (Table 2). IR values were adjusted for age, BMI, and WHR by regression equation and divided into tertiles. Concentrations of metabolic variables in these tertiles were compared by ANOVA (Table 3). Multiple linear regression analysis with stepwise addition of BMI, fasting and 2 h SI, and fasting and 2 h PI as independent variables was done using log leptin as the

Table 1. Clinical and biochemical details of the study group

Parameters	Men	Women
Number	80	64
Age (yr)	45 ± 11	42 ± 13
BMI (kgm^{-2})	23.0 ± 4.0	24.4 ± 3.2
WHR	0.89 ± 0.07	0.84 ± 0.06[b]
Plasma glucose (mmol l^{-1})		
Fasting	5.7 ± 0.65	5.6 ± 0.65
2 h	6.4 ± 1.6	6.5 ± 1.4
Specific insulin (pmol l^{-1})[a]		
Fasting	59.5 (57.4–61.6)	60.4 (58–62.8)
2 h	223 (220.8–225.2)	252 (249.4–254.6)
Proinsulin (pmol l^{-1})[a]		
Fasting	5.7 (3.6–7.8)	5.8 (4.6–7.0)
2 h	21.8 (18.1–25.5)	20.5 (18.5–22.5)
IR[a]	3.0 (0.9–5.1)	3.1 (1.0–5.2)
BF[a]	123.2 (121–125.3)	119.6 (117.5–121.7)
Leptin (ng ml^{-1})[a]	5.1 (3.0–7.2)	17.9[b] (15.4–20.3)

[a]Geometric mean, other values are mean ± SD.
Values in brackets are 95 % confidence intervals. [b]$p<0.05$ compared to men.
IR, insulin resistance (HOMA), BF, β-cell function (HOMA).

Table 2. Partial correlation of IR (log) with anthropometric and metabolic variables

Variables	Dependent variables IR (log)			
	Men		Women	
	r	*p*	*r*	*p*
Age	–0.009	0.94	0.219	0.12
BMI	0.115	0.36	0.054	0.70
WHR	–0.063	0.62	–0.062	0.66
Plasma glucose				
2 h	–0.001	0.99	0.290	0.04
Specific Insulin (log)				
2 h	0.225	0.07	0.062	0.66
Proinsulin (log)				
Fasting	0.266	0.03	0.369	0.01
2 h	0.025	0.85	–0.078	0.58
Log Leptin	–0.067	0.60	–0.166	0.24
BF (log)	0.61	0.000	0.658	0.000

IR, insulin resistance (HOMA); BF, β-cell function (HOMA).

Table 3. Distribution of the metabolic variables in the tertiles of IR (adjusted for age, BMI, WHR)

	Tertiles of IR		
	1	2	3
IR (range)	0.44–1.96	>1.96–3.29	>3.29
Specific insulin (pmol l^{-1})			
Fasting	32.6 (30.5–34.7)	59.6[a] (57.6–61.6)	111.8[b,c] (109.7–113.9)
2 h	153.5 (151.2–155.8)	219.7 (217.5–221.9)	391.2[b,c] (389.0–393.4)
Proinsulin (pmol l^{-1}			
Fasting	4.0 (2.1–5.94)	4.8 (2.78–6.82)	9.7[b,c] (7.58–11.82)
2 h	15.2 (13–17.42)	17.1 (14.87–19.33)	36[b,c] (33.8–38.2)
Leptin			
Men	3.3 (0.76–5.84)	5.2 (2.8–7.6)	7.4[b,c] (4.8–10)
Women	16.5 (13.6–19.42)	18.8 (14.9–22.7)	19.0 (15.8–22.2)
BF			
Men	50.5 (46.7–54.3)	90.9[a] (86.2–95.6)	164.6[b,c] (146.3–183)
Women	73.8 (68.7–78.9)	87.7 (83.3–92.1)	154.8[b,c] (149.3–160.3)

Geometric means are shown. Values in brackets 95 % confidence intervals.
[a,b,c]Denote $p<0.05$: [a] 2 vs 1, [b] 3 vs 1, [c] 3 vs 2.
ANOVA was carried out using log transformed mean and SD values. IR, insulin resistance (HOMA); BF, β-cell function (HOMA).

dependent variable, where age, BMI, and WHR were forced to enter the equation due to their confounding nature. Tests were done separately in men and women. Fasting SI was in categories of 50 pmol l^{-1} and 2 h SI in categories of 100 pmol l^{-1} (Table 4). Associations of age, BMI, WHR, glucose tolerance (NGT and IGT), PI, 2 h SI, BF, and leptin with IR were tested by multiple regression analysis by stepwise addition method in men and women separately. Here also age, BMI, and WHR were forced into the equations (Table 5). Log transformation of the variables were done as in Table 1. SPSS PC version 4.0.1 was used for statistical analysis.

Table 4. Multiple linear regression analyses using log transformed leptin as the dependent variable (stepwise forward method). Independent variables included were: age, BMI, WHR, specific insulin—fasting and 2 h, proinsulin—fasting and 2 h. Age, BMI, and WHR were forced into the model

Significant independent variables	β	SE(β)	P	Total R^2(%)
Male				
Age	–0.0017	0.0069	0.80	
BMI	0.089	0.019	0.0001	56.2
WHR	2.69	1.00	0.01	
Specific insulin—2 h	0.109	0.037	0.005	
Female				
Age	–0.0084	0.0038	0.03	
BMI	0.0726	0.0143	0.0001	40.1
WHR	–0.889	0.719	0.22	

Specific insulin—Fasting values in categories of 50 pmol l^{-1}, 2 h values in categories of 100 pmol l^{-1}.

Results

Clinical and the metabolic characteristics of men and women in the study are shown in Table 1. Women had lower mean WHR than men. All biochemical parameters were similar in men and women, except leptin which was significantly higher in women.

Partial correlations of log IR with the variables studied are shown in Table 2. In men, fasting proinsulin (log) and log BF showed positive correlations with IR while in women 2 h plasma glucose and BF (log) were correlated to IR. Leptin was not correlated to IR.

Table 3 shows the distribution of the metabolic variables in relation to the tertiles of IR, corrected for age, BMI, and WHR. As expected, SI values increased with increasing tertiles. Proinsulin concentrations were high only in the 3rd tertile. In men, leptin concentration was high in the 3rd tertile in comparison with the other tertiles. Women showed no such differences with increasing tertiles. In men, BF increased with increasing tertiles, while in women higher BF values were seen in the 3rd tertile only.

Associations of leptin (log) with fasting and 2 h plasma specific insulin and proinsulin concentration (categorized) and age, BMI, and WHR (all three forced into the equation) were tested by multiple linear regression analysis. The variations in leptin concentrations in men were associated with BMI, WHR ($R^2 = 50.6$ %) and 2 h

Table 5. Result of multiple linear regression analyses: dependent variable = IR (log); Independent variables included in the test: age, BMI, WHR, diagnosis (NGT and IGT); log SI-2h, log PI-F and 2h, log leptin, log BF. Age, BMI, and WHR were forced into the model

Significant Independent Variables	β	SE(β)	P	Total R^2 %
Men				
Age	0.0011	0.005	0.828	
BMI	0.0123	0.0162	0.4524	
WHR	0.567	0.799	0.4803	66.9
Log BF	0.5605	0.0738	<0.001	
Log PI—2h	0.1964	0.0594	0.0015	
Women				
Age	0.0126	0.0052	0.0188	
BMI	-3.90^{-04}	0.0177	0.9825	
WHR	–2.185	0.883	0.0168	61.3
Log BF	0.755	0.100	<0.001	
Log SI—2h	0.153	0.071	0.0349	

PI, proinsulin; SI, specific insulin.

SI values ($R^2 = 5.6$ %), while fasting SI and proinsulin concentrations had no significant contributions. A similar analysis in women showed that age and BMI showed significant association with serum leptin values ($R^2 = 40.1$ %) (Table 4).

Table 5 shows the results of multiple linear regression analysis in men and women separately, using IR (log) as the dependent variable. Age, BMI, WHR, diagnosis (NGT and IGT), log BF, log fasting PI, log 2 h PI, log 2 h SI, and log of leptin were included as independent variables using a stepwise addition method. The first three variables were forced into the equation. In men, BF and 2 h PI were correlated ($R^2 = 66.9$ %). In women BF ($R^2 = 61.3$ %) and 2 h SI, age, and WHR showed significant correlation with IR. Leptin did not show an independent association with IR in either sex.

Discussion

The important outcome of this analysis is that it confirms the earlier observation that plasma leptin and insulin concentration are only weakly associated in Asian Indian subjects.[5] Use of a non-specific measure of insulin did not cause a significant change in interpretation of the data. Non-specific insulin explained 2.4 % of the variations in leptin. Using a specific insulin assay, 5.6 % of the variation in plasma leptin were explained, only in men. This lack of a strong correlation between the two variables in Asian Indians is different from the observations in Western Samoans,[1] in Swedish women,[2] and also from two other reports in White populations.[3,4] In all these groups, a strong independent correlation between fasting insulin and leptin was observed, thereby suggesting a possible role for leptin in insulin resistance.[1–4] Ethnic variation is thus evident in the association between leptin and plasma insulin values, the nature of which is unknown. Asian Indians, who have higher insulin responses than white populations,[12–17] do not have higher leptin values.

Leptin did not show an association with proinsulin concentration or with insulin resistance. As expected, in men and women, BF and IR were strongly related. The other variables independently associated with IR were 2 h PI in men and 2 h SI in women, both of which could be secreted in higher concentrations to compensate for increased insulin resistance. Blood concentration of PI also increases simultaneously with SI when more of insulin is required for biological functions.[18,19]

IR had an independent association with BF as indicated by higher BF values in increasing tertiles of IR. As we had included no frankly diabetic subjects in the study, the enhanced beta-cell secretion compensatory to increased insulin resistance was evident. The lack of a significant independent correlation between insulin resistance and leptin in this population requires explanation.

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